METHOD 8318A

N-METHYLCARBAMATES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

1.0 SCOPE AND APPLICATION

1.1 Method 8318 is used to determine the concentration of *N*-methylcarbamates in soil, water, and waste matrices. The following compounds have been determined by this method:

Compound Name	CAS No.[a]
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Bendiocarb	22781-23-3
Carbaryl (Sevin)	63-25-2
Carbofuran (Furadan)	1563-66-2
m-Cumenyl methylcarbamate	64-00-6
Dioxacarb	6988-21-2
Formetanate hydrochloride	23422-53-9
3-Hydroxycarbofuran	16655-82-6
Methiocarb (Mesurol)	2032-65-7
Methomyl (Lannate)	16752-77-5
Metolcarb	1129-41-5
Mexacarbate	315-18-4
Oxamyl	23135-22-0
Promecarb	2631-37-0
Propoxur (Baygon)	114-26-1
Thiodicarb	59669-26-0

[a]Chemical Abstracts Service Registry Number

1.2 This method provides optional matrix-specific extraction procedures that can be applied to aqueous samples, soils, solids, sludges, and oily wastes. In addition, optional extract cleanup procedures are included to remove interferences and improve method sensitivity.

1.3 The sensitivity of the method usually depends on the level of interferences present, rather than on the instrumental conditions. Waste samples with a high level of extractable fluorescent compounds are expected to yield significantly higher detection limits.

1.4 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500,

3600, 5000, and 8000) for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two, Sec. 2.1, for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

1.5 This method is restricted to use by, or under the supervision of, analysts experienced in the use of high performance liquid chromatography (HPLC) and skilled in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 *N*-methylcarbamates may be extracted from samples using various 3500 Series extraction methods, or other methods that are appropriate for the sample matrix. Alternatively, several optional matrix-specific extraction procedures are provided in this method. Those optional procedures include:

2.1.1 Extraction of 100-mL volumes of aqueous samples with methylene chloride in a separatory funnel.

2.1.2 Extraction of soils, solids, sludges, and heavy aqueous suspensions with acetonitrile on a platform shaker.

2.1.3 Extraction of soils that are heavily contaminated with oils or other non-aqueous liquids with hexane, followed by acetonitrile, on a platform shaker.

2.2 Extracts may be subjected to an optional cleanup procedure designed to remove interferences. During cleanup, the solvent is exchanged to methanol for analysis.

2.3 Extracts are analyzed by HPLC, on a C-18 reversed-phase column. After separation, the target analytes are hydrolyzed and derivatized post-column, then quantitated fluorometrically.

2.4 Due to the specific nature of this analysis, confirmation by a secondary method is not essential. However, fluorescence due to post-column derivatization may be confirmed by substituting the NaOH and *o*-phthalaldehyde solutions with reagent water and reanalyzing the sample. If fluorescence is still detected, then a positive interference is present and care should be taken in the interpretation of the results.

3.0 INTERFERENCES

3.1 Fluorescent compounds, primarily alkyl amines and compounds which yield primary alkyl amines upon base hydrolysis, are potential sources of interferences.

3.2 Coeluting compounds that are fluorescence quenchers may result in negative interferences.

3.3 Impurities in solvents and reagents are additional sources of interferences. Before processing any samples, the analyst must demonstrate daily, through the analysis of solvent blanks, that the entire analytical system is interference free.

4.0 APPARATUS AND MATERIALS

4.1 High performance liquid chromatograph (HPLC)

An analytical system with a programmable solvent delivery system and all necessary accessories including a high pressure injection valve, analytical column, mobile phase solvent degassing, etc. The solvent delivery system must be capable, at a minimum, of handling a binary solvent system, and must be able to accurately deliver flow rates between 0.5 - 3.0 mL/min.

4.1.1 HPLC columns - An analytical column is required and a guard column is highly recommended.

4.1.1.1 C-18 reversed-phase column, 3-5 µm particle size, 100 - 250 mm x 4.6-mm ID.

4.1.1.2 Guard column - Packing and internal diameter similar to that used in the analytical column.

4.1.2 Post-column reactor with two solvent delivery systems.

4.1.3 Fluorescence detector - Capable of excitation at 280 nm and emission greater than 389 nm cutoff, at a minimum.

4.1.4 Column heater - The use of a column heater is recommended to ensure consistent retention times throughout the course of the HPLC analysis. The heater should be capable of maintaining the analytical column at 3 to 5°C (± 0.1°C) above the ambient laboratory temperatures.

4.1.5 Autoinjector - The use of an autoinjector is recommended. The autoinjector should be capable of delivering 1 - 25 µL injections without affecting the chromatography.

4.1.6 HPLC injection syringe - 50-µL, used if the autoinjector is not used.

4.1.7 Data system - At a minimum, an integrator compatible with the detector, and capable of measuring peak area and retention time for the eluted peaks. A computer system for instrumental control, data collection and analysis is recommended.

4.2 Analytical balance capable of weighing ± 0.0001 g.

4.3 Top loading balance capable of weighing ± 0.01 g.

4.4 Volumetric pipettes, Class A - Glass, assorted sizes.

4.5 Volumetric flasks, Class A - Glass, assorted sizes.

4.6 Assorted glass funnels.

4.7 Equipment for the optional extraction procedures

The following equipment is used for the optional extraction procedures described in this method.

4.7.1 Centrifuge capable of holding 250-mL tubes.

4.7.2 Platform shaker capable of holding 250-mL Erlenmeyer flasks.

4.7.3 Erlenmeyer flasks with PTFE-lined screw caps, 250-mL.

4.7.4 Filter paper, (Whatman #113 or #114, or equivalent).

4.7.5 Separatory funnels, with ground-glass stoppers and stopcocks - 250-mL.

4.7.6 Graduated cylinders - 100-mL.

4.7.8 Centrifuge tubes - 250-mL.

4.7.9 Vials - 25-mL, glass with PTFE-lined screw caps or crimp tops.

4.8 Equipment for the optional cleanup techniques

4.8.1 Heating block, or equivalent apparatus, that can accommodate 10-mL graduated vials (see Sec. 4.8.2).

4.8.2 Graduated glass vials - 10-mL, 20-mL.

4.8.3 Reversed-phase cartridges, (Waters Associates C-18 Sep-Pak®, or equivalent).

4.8.4 Nylon filter unit, 25-mm diameter, 0.45-µm pore size, disposable.

5.0 REAGENTS

5.1 HPLC grade chemicals shall be used in all tests. All reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination.

5.2 Solvents

Some of the following solvents may not be needed if the optional extraction and cleanup procedures are not employed.

5.2.1 Acetonitrile, CH_3CN - HPLC grade - minimum UV cutoff at 230 nm.

5.2.2 Methanol, CH_3OH - HPLC grade - minimum UV cutoff at 230 nm.

5.2.3 Methylene chloride, CH_2Cl_2 - HPLC grade - minimum UV cutoff at 230 nm.

5.2.4 Hexane, C_6H_{14} - pesticide grade.

5.2.5 Ethylene glycol, $HOCH_2CH_2OH$ - reagent grade.

5.3 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.4 Sodium hydroxide, NaOH - reagent grade - 0.05N NaOH solution.

5.5 Phosphoric acid, H_3PO_4 - reagent grade.

5.6 Borate buffer, pH 10.

5.7 *o*-Phthalaldehyde, *o*-$C_6H_4(CHO)_2$ - reagent grade.

5.8 2-Mercaptoethanol, $HSCH_2CH_2OH$ - reagent grade.

5.9 *N*-methylcarbamate neat standards.

5.10 Chloroacetic acid, $ClCH_2COOH$, 0.1 N.

5.11 Reaction solution

Dissolve 0.500 g of *o*-phthalaldehyde in 10 mL of methanol, in a 1-L volumetric flask. To this solution, add 900 mL of organic-free reagent water, followed by 50 mL of the borate buffer (pH 10). After mixing well, add 1 mL of 2-mercaptoethanol, and dilute to the mark with organic-free reagent water. Mix the solution thoroughly. Prepare fresh solutions on a weekly basis, as needed. Protect from light and store under refrigeration.

5.12 Standard solutions

The following sections describe the preparation of stock, intermediate, and working standards for the compounds of interest. This discussion is provided as an example, and other approaches and concentrations of the target compounds may be used, as appropriate for the intended application. See Method 8000 for additional information on the preparation of calibration standards.

5.12.1 Stock standard solutions

Prepare individual 1000 mg/L solutions by adding 0.025 g of carbamate to a 25-mL volumetric flask, and diluting to the mark with methanol. Store solutions, under refrigeration, in glass vials with PTFE-lined screw caps or crimp tops. Replace every six months, or sooner if necessary.

5.12.2 Intermediate standard solution

Prepare a solution containing all the target compounds at 50.0 mg/L by adding 2.5 mL of each stock solution to a 50-mL volumetric flask, and diluting to the mark with methanol. Store solutions, under refrigeration, in glass vials with PTFE-lined screw caps or crimp tops. Replace every three months, or sooner if necessary.

5.12.3 Working standard solutions

Prepare 0.5, 1.0, 2.0, 3.0 and 5.0 mg/L solutions by adding 0.25, 0.5, 1.0, 1.5 and 2.5 mL of the intermediate mixed standard to respective 25-mL volumetric flasks, and diluting each to the mark with methanol. Store solutions, under refrigeration, in glass vials with PTFE-lined screw caps or crimp tops. Replace every two months, or sooner if necessary.

NOTE: Other concentrations may be used as appropriate for the intended application and the operating range of the instrument.

5.12.4 Mixed QC standard solution

Prepare a 40.0 mg/L solution from another set of stock standard solutions, prepared similarly to those described in Sec. 5.12.1. Add 2.0 mL of each stock solution to a 50-mL volumetric flask and dilute to the mark with methanol. Store the solution, under refrigeration, in a glass vial with a PTFE-lined screw cap or crimp top. Replace every three months, or sooner if necessary. Other concentrations may be used, as appropriate for the intended application.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Due to the extreme instability of *N*-methylcarbamates in alkaline media, samples of water, wastewater, and leachates should be preserved immediately after collection by acidifying to pH 4-5 with 0.1 N chloroacetic acid.

6.2 Store samples at 4 ± 2°C, and out of direct sunlight, from the time of collection through analysis. *N*-methylcarbamates are sensitive to alkaline hydrolysis and heat.

6.3 All samples must be extracted within seven days of collection, and should be analyzed within 40 days of extraction.

7.0 PROCEDURE

Samples may be prepared and extracted using various 3500 Series extraction methods or other methods that are appropriate for the sample matrix. Alternatively, optional matrix-specific extraction procedures are provided in this method. These optional procedures use smaller volumes or weights of samples than are nominally employed in the 3500 Series methods (e.g., a 100-mL water sample versus a 1-L water sample). The analyst should choose among the possible extraction procedures based on the nature of the samples and the sensitivity required for the intended application. This method also includes optional cleanup and solvent exchange procedures.

Whatever extraction procedure is employed, *including* those specifically listed in this method, the analyst *must* demonstrate adequate performance for the analytes of interest, at the levels of interest. At a minimum, such a demonstration will encompass the initial demonstration of proficiency described in Sec. 8.2 of Method 3500, using a clean reference matrix. Method 8000 describes procedures that may be used to develop performance criteria for such demonstrations as well as for matrix spike and laboratory control sample results.

7.1 Extraction of water, wastewater, aqueous industrial wastes, and leachates

Refer to Chapter Two and Method 3500 for guidance in choosing the appropriate extraction procedure. In general, water samples are extracted at a neutral pH with methylene chloride using a separatory funnel (Method 3510), a continuous liquid-liquid extractor (Method 3520), or other appropriate technique. If one of these techniques is employed, then proceed to Sec. 7.5 once the extraction is complete.

Alternatively, if appropriate for the sensitivity of the intended application, use the approach described below.

7.1.1 Measure 100 mL of sample into a 250-mL separatory funnel.

7.1.2 Add 30 mL of methylene chloride to the separatory funnel, cap, and shake vigorously for about 2 minutes. Allow the phases to separate and drain the organic layer into a clean 100-mL volumetric flask. Repeat the extraction two more times with fresh portions of solvent.

7.1.3 Combine all three extracts in a 100-mL volumetric flask and dilute to 100 mL with methylene chloride. Proceed to Sec. 7.5.

7.2 Extraction of soils, solids, sludges, and heavy aqueous suspensions

Refer to Chapter Two and Method 3500 for guidance in choosing the appropriate extraction procedure. In general, solid samples are extracted with acetonitrile using one of the Soxhlet extraction methods (Method 3540 or 3541), pressurized fluid extraction (Method 3545), microwave extraction (Method 3546), ultrasonic extraction (Method 3550), or other appropriate technique or solvents. If one of these techniques is employed, then proceed to Sec. 7.5 once the extraction is complete.

Alternatively, if appropriate for the sensitivity of the intended application, use the approach described below.

7.2.1 Weigh out 20 ± 0.1 g of sample into a 250-mL Erlenmeyer flask with a PTFE-screw cap.

7.2.2 Determination of percent dry weight - When sample results are to be calculated on a dry weight basis, a second portion of sample should be weighed out at the same time as the portion used for analytical determination.

WARNING: The drying oven should be contained in a hood or vented. Significant laboratory contamination may result from drying a heavily contaminated sample.

Immediately after weighing the sample for extraction, weigh 5-10 g of the sample into a tared crucible. Dry this aliquot overnight at 105°C. Allow it to cool in a desiccator before weighing. Calculate the % dry weight as follows:

$$\%\ \text{dry weight} = \frac{\text{g of dry sample}}{\text{g of sample}} \times 100$$

NOTE: This dry weight determination also applies to the use of other extraction techniques for solids (e.g., a 3500 Series method), when results are to be calculated on a dry weight basis.

7.2.3 Add 50 mL of acetonitrile to the portion of the sample in the Erlenmeyer flask. Seal the flask and shake for 2 hours on a platform shaker. Allow the mixture to settle (5-10 min), then decant the extract into a 250-mL centrifuge tube. Repeat the extraction twice more, but using 20 mL of acetonitrile and shaking for 1 hour each time.

7.2.4 Combine all three extracts in the centrifuge tube. Centrifuge the combined extract at 200 rpm for 10 min. Carefully decant the supernatant into a 100-mL volumetric flask and dilute to 100 mL with acetonitrile. Proceed to Sec. 7.5.

7.3 Soils heavily contaminated with non-aqueous substances, such as oils

The extraction of soils heavily contaminated with oils and other non-aqueous substances is not generally addressed in the various 3500 Series extraction methods for solid samples. Therefore, the procedure outlined below may be more appropriate for such samples. However the choice of extraction procedure is left to the analyst.

7.3.1 Weigh out 20 ± 0.1 g of sample into a 250-mL Erlenmeyer flask with a PTFE-screw cap.

7.3.2 If dry weight results are required, determine the dry weight as described in Sec. 7.2.2.

7.3.3 Add 60 mL of hexane to the portion of the sample in the Erlenmeyer flask. Seal the flask and shake for 1 hour on a platform shaker. Without removing the hexane, add 50 mL of acetonitrile and shake for an additional 3 hours. Allow the mixture to settle (5-10 min), then decant the solvent layers into a 250-mL separatory funnel, leaving the sample in the Erlenmeyer flask. Drain the acetonitrile (bottom layer) through filter paper into a 100-mL volumetric flask.

7.3.4 Add 60 mL of hexane and 50 mL of acetonitrile to the original Erlenmeyer flask and shake for an additional 1 hour. Allow the mixture to settle, then decant the solvent mixture into the separatory funnel containing the hexane from the first extraction. Shake the separatory funnel for 2 minutes, allow the phases to separate, drain the acetonitrile layer through filter paper into the volumetric flask.

7.3.5 Dilute to 100 mL with acetonitrile. Proceed to Sec. 7.5.

7.4 Non-aqueous liquids such as oils

The extraction of oils and other non-aqueous liquids is not generally addressed in the various 3500 Series extraction methods. Therefore, the procedure outlined below may be more appropriate for such samples. However the choice of extraction procedure is left to the analyst.

7.4.1 Weigh out 20 ± 0.1 g of sample into a 125-mL separatory funnel. Add 40 mL of hexane and 25 mL of acetonitrile and vigorously shake the sample mixture for 2 minutes. Allow the phases to separate, then drain the acetonitrile (bottom layer) into a 100-mL volumetric flask.

7.4.2 Add another 25 mL of acetonitrile to the separatory funnel and shake for 2 minutes. Allow the phases to separate and drain the acetonitrile layer into the volumetric flask.

7.4.3 Repeat the extraction with a third 25-mL portion of acetonitrile, combining the extracts in the volumetric flask.

7.4.4 Dilute to 100 mL with acetonitrile. Proceed to Sec. 7.5.

7.5 Cleanup and solvent exchange

This section provides optional cleanup procedures for the extracts from all matrices. While extracts of some very clean water samples may not require cleanup, the use of the cleanup procedures is strongly recommended for water samples and may be essential for the other matrices. These cleanup procedures assume that each sample extract begins with a volume of 100 mL. The volumes described in the cleanup procedures are recommendations, and the analyst is free to employ other volumes, provided that the analyst demonstrates acceptable performance for the intended application. Regardless of the specific volumes that are employed, the analyst must record the volumes at each step, so that the overall dilution factor for the analysis can be determined and the concentration of each target compound can be calculated.

All sample extracts will require solvent exchange prior to analysis. The optional cleanup techniques include a solvent exchange step. However, if the cleanup steps are omitted, proceed to Sec.7.5.3 for solvent exchange.

7.5.1 Cleanup of water sample extracts in methylene chloride

Prior to cleanup, the solvent must be exchanged from methylene chloride to a solvent compatible with the C-18 cleanup cartridge (e.g., methanol). After cleanup, the extract is filtered.

7.5.1.1 Pipet 20.0 mL of the 100-mL extract into a 20-mL glass vial containing 100 µL of ethylene glycol. Place the vial in a heating block set at 50°C, and gently evaporate the extract under a stream of nitrogen (in a fume hood) until only the ethylene glycol keeper remains. Retain the remaining 80 mL of methylene chloride extract in the volumetric flask in the event it is need for dilutions or reanalysis.

7.5.1.2 Dissolve the ethylene glycol residue in 2 mL of methanol.

7.5.1.3 Pass the 2 mL of methanol through a C-18 reversed-phase cartridge, pre-washed with methanol. Collect the eluate in a 5-mL volumetric flask. Elute the cartridge with about 3 mL of methanol, and collect the eluate until a final volume of 5.0 mL is obtained.

7.5.1.4 Using a disposable 0.45-µm filter, filter the cleaned extract directly into a labeled autosampler vial or other suitable container. The extract is now ready for analysis. Proceed to Sec. 7.7. The 5.0-mL volume of methanol represents one-fifth (20/100) of the original sample extract.

7.5.2 Cleanup of acetonitrile extracts of soils, solids, sludges, heavy aqueous suspensions, and non-aqueous liquids

Acetonitrile is compatible with the C-18 cartridges used for cleanup. Therefore, the solvent exchange step occurs after the cleanup, unlike the methylene chloride extracts of aqueous samples described above.

7.5.2.1 Pass 15 mL of the 100-mL acetonitrile extract through a C-18 reversed-phase cartridge, prewashed with 5 mL of acetonitrile. Discard the first 2 mL of eluate and collect the remainder in a clean flask. Retain the remaining 85 mL of acetonitrile extract in the volumetric flask in the event it is need for dilutions or reanalysis.

7.5.2.2 Pipet 10.0 mL of the cleaned extract into a 10-mL graduated glass vial containing 100 µL of ethylene glycol. Place the vial in a heating block set at 50°C, and gently evaporate the extract under a stream of nitrogen (in a fume hood) until only the ethylene glycol keeper remains.

7.5.2.3 Add methanol to the ethylene glycol residue, dropwise, until the total volume is 1.0 mL.

7.5.2.4 Using a disposable 0.45-µm filter, filter this extract directly into a labeled autosampler vial or other suitable container. The extract is now ready for analysis. Proceed to Sec. 7.7. The 1.0-mL volume of methanol represents one-tenth (10/100) of the original sample extract.

7.5.3 Solvent exchange for extracts that do not undergo cleanup

The final extract solvent must be compatible with the HPLC column. Therefore, if the cleanup steps in Sec. 7.5.2 have been omitted, the analyst must exchange the solvent to methanol or another solvent compatible with the HPLC system. The solvent exchange may be accomplished using the ethylene glycol keeper approach described above, or by

other appropriate means, including those described in the 3500 Series methods themselves.

7.6 HPLC conditions

Establish HPLC operating conditions appropriate for the target analytes. Optimize the instrumental conditions for resolution of the target analytes and sensitivity. Suggested operating conditions are given below. Other operating conditions may be employed, provided that the analyst can demonstrate performance that is appropriate for the intended application. Table 2 provides the example retention times that were obtained under these conditions during method development. These retention times are for illustrative purposes only. Each laboratory must determine retention times for its specific application of the method. An example chromatogram is shown in Figure 1.

NOTE: Once established, the same operating conditions must be used for both calibrations and sample analyses.

7.6.1 Recommended solvents and flow rate

Solvent A:	Reagent water, acidified with 0.4 mL of phosphoric acid per liter of water
Solvent B:	Methanol/acetonitrile (1:1, v/v)
Flow rate:	1.0 mL/min
Injection volume:	20 µL

7.6.2 Recommended gradient elution program

Time (min)	Solvent A (%)	Solvent B (%)
0.00	90%	10%
0.02	20%	80%
20.02	0%	100%
25.02	0%	100%
30.02	90%	10%
33.02	90%	10%

7.6.3 Recommended post-column hydrolysis parameters

Solution:	0.05 N aqueous sodium hydroxide
Flow rate:	0.7 mL/min
Temperature:	95°C
Residence time:	35 seconds (1-mL reaction coil)

7.6.4 Recommended post-column derivatization parameters

Solution:	*o*-phthalaldehyde/2-mercaptoethanol
Flow rate:	0.7 mL/min
Temperature:	40°C
Residence time:	25 seconds (1-mL reaction coil)

7.6.5 Recommended fluorometer parameters

Cell:	10 µL
Excitation wavelength:	340 nm
Emission wavelength:	418 nm cutoff filter
Sensitivity wavelength:	0.5 µA
PMT voltage:	-800 V
Time constant:	2 sec

7.7 Calibration

7.7.1 Analyze a solvent blank (20 µL of methanol) to ensure that the system is clean. Analyze the calibration standards (see Sec. 5.12.3), starting with the lowest standard and ending with the highest standard, to avoid memory effects.

7.7.2 Calculate the calibration factor (CF) for each analyte at each concentration as:

$$CF = \frac{\text{Peak Area (or Height) of the Compound in the Standard}}{\text{Mass of the Compound Injected (in nanograms)}}$$

7.7.3 Calculate the mean calibration factor for each analyte as:

$$\text{mean CF} = \overline{CF} = \frac{\sum_{i=1}^{n} CF_i}{n}$$

where n is the number of standards analyzed.

7.7.4 Calculate the standard deviation (SD) and the RSD of the calibration factors for each analyte as:

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (CF_i - \overline{CF})^2}{n-1}} \qquad RSD = \frac{SD}{\overline{CF}} \times 100$$

7.7.5 Calibration linearity

If the RSD for each analyte is ≤ 20%, then the response of the instrument is considered linear and the mean calibration factor may be used to quantitate sample results. If the RSD is greater than 20%, the analyst should consult Method 8000 for other calibration options, which may include: the grand mean RSD approach, a linear calibration not through the origin, or a non-linear calibration model (e.g., a polynomial equation).

7.7.6 Retention time windows

Method 8000 provides instructions on establishing retention time windows and identification criteria. If column temperature control is not employed, special care must be taken to ensure that temperature shifts do not cause peak misidentification.

7.8 Sample analysis

Analyze the sample extracts using the same HPLC operating conditions that were established in Sec. 7.6.

7.8.1 Each sample analysis must be bracketed with an acceptable initial calibration, calibration verification standard(s) (each 12-hour analytical shift), or calibration standards interspersed within the samples.

7.8.2 The calibration factor for each analyte should not exceed a ± 15 percent difference from the mean calibration factor calculated for the initial calibration. If a calibration approach other than the RSD method has been employed for the initial calibration (e.g., a linear model not through the origin, a non-linear calibration model, etc.), consult Sec. 7 of Method 8000 for the specific details of calibration verification.

The percent difference is calculated as:

$$\% \text{ Difference} = \frac{CF - \overline{CF}_v}{\overline{CF}} \times 100$$

7.8.3 If this criterion is exceeded for any analyte, use the approach described in Sec. 7 of Method 8000 to calculate the average percent difference across all analytes. If the average of the responses for all analytes is within ±15%, then the calibration has been verified. However, the conditions in Sec. 7 of Method 8000 also apply, e.g., the average must include all analytes in the calibration, regardless of whether they are target analytes for a specific project, and the data user must be provided with the calibration verification data or a list of those analytes that exceeded the ±15% limit.

7.8.4 If the calibration does not meet the ±15% limit (either on the basis of each compound or the average across all compounds), check the instrument operating conditions, and if necessary, restore them to the original settings, and inject another aliquot of the calibration verification standard. If the response for the analyte is still not within ±15%, then a new initial calibration must be prepared.

7.8.5 When a calibration verification standard fails to meet the QC criteria, all samples that were injected after the last standard that last met the QC criteria must be evaluated to prevent misquantitations and possible false negative results, and reinjection of the sample extracts may be required. More frequent analyses of standards will minimize the number of sample extracts that would have to be reinjected if the QC limits are violated for the standard analysis.

However, if the standard analyzed after a group of samples exhibits a response for an analyte that is above the acceptance limit, i.e., >15%, and the analyte was not detected in the specific samples analyzed during the analytical shift, then the extracts for those samples do not need to be reanalyzed, as the verification standard has demonstrated that the analyte would have been detected were it present. In contrast, if an analyte above the QC limits was detected in a sample extract, then reinjection is necessary to ensure accurate quantitation. If an analyte was not detected in the sample and the standard response is more than 15% below the initial calibration response, then reinjection is necessary to ensure that the detector response has not deteriorated to the point that the analyte would not have been detected even though it was present (i.e., a false negative result).

7.8.6 Using the retention time windows established during calibration, identify the peaks in the sample chromatogram.

7.9 Calculation of sample results

The calculation of the concentration of each analyte identified in the sample generally follows the procedures outlined in Method 8000 for external standard calibration. However, because the optional matrix-specific extraction and cleanup procedures described in this method involve various dilutions, the equations for the calculations are provided below in detail.

If the optional extraction and cleanup procedures are not employed, then the analyst should consult Method 8000 and adjust the calculations accordingly.

7.9.1 Aqueous samples

$$\text{Concentration } (\mu g/L) = \frac{(A_x)(V_e)(V_t)(D)}{(\overline{CF})(V_s)(V_c)(V_i)}$$

where:

A_x = Area (or height) of the peak for the analyte in the sample.

V_e = Total volume of the original extract (mL). For aqueous samples extracted using the procedure in Sec. 7.1, this value will be 100 mL.

V_c = Volume of the extract processed through the cleanup steps (mL). For aqueous samples processed through the optional cleanup steps, this value will be 20 mL.

V_t = Total volume of the concentrated methanol extract (µL). For aqueous samples processed through the optional cleanup steps, this volume will be 5000 µL

D = Dilution factor, if the sample or extract was diluted prior to analysis. If no dilution was made, D = 1. The dilution factor is always dimensionless. This dilution factor is in addition to the inherent dilution that occurs during the optional cleanup steps, e.g., it is *not* used to account for V_e/V_c.

$\overline{CF}$ = Mean calibration factor from the initial calibration (area/ng).

V_i = Volume of the extract injected (µL). The injection volume for samples and calibration standards should be the same, unless the analyst can demonstrate acceptable performance using different volumes or conditions.

V_s = Volume of the aqueous sample extracted in mL. If units of liters are used for this term, multiply the results by 1000.

Using the units specified here for these terms will result in a concentration in units of ng/mL, which is equivalent to µg/L.

7.9.2 Non-aqueous samples

$$\text{Concentration (µg/kg)} = \frac{(A_x)(V_e)(V_t)(D)}{(\overline{CF})(W_s)(V_c)(V_i)}$$

where:

A_x = Area (or height) of the peak for the analyte in the sample.

V_e = Total volume of the original extract (mL). For most non-aqueous samples extracted using the procedure in Sec. 7.2, this value will be 100 mL.

V_c = Volume of the extract *recovered* from the cleanup steps (mL). For most non-aqueous samples processed through the optional cleanup steps, this value will be 10 mL, even though 15 mL of the original extract are passed through the C-18 cartridge.

V_t = Total volume of the concentrated methanol extract (µL). For most non-aqueous samples processed through the optional cleanup steps, this volume will be 1000 µL

D = Dilution factor, if the sample or extract was diluted prior to analysis. If no dilution was made, D = 1. The dilution factor is always dimensionless. This dilution factor is in addition to the inherent dilution that occurs during the optional cleanup steps, e.g., it is *not* used to account for V_e/V_c.

$\overline{CF}$ = Mean calibration factor from the initial calibration (area/ng).

V_i = Volume of the extract injected (µL). The injection volume for samples and calibration standards should be the same, unless the analyst can demonstrate acceptable performance using different volumes or conditions.

V_s = Volume of the non-aqueous sample extracted in g. If units of kilograms are used for this term, multiply the results by 1000.

Using the units specified here for these terms will result in a concentration in units of ng/g, which is equivalent to µg/kg.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. The quality control procedures to ensure the proper operation of the various sample preparation techniques can be found in Method 3500 and can be applied to this method, which contains its own extraction procedures. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 The quality control procedures necessary to evaluate the HPLC system operation are found in Method 8000, Sec. 7.0 and include evaluation of retention time windows, calibration verification, and chromatographic analysis of samples.

8.3 Initial demonstration of proficiency

8.3.1 Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made.

8.3.2 Refer to Sec. 8.0 of Method 8000 for procedures for evaluating method performance.

8.4 Sample quality control for preparation and analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision and accuracy). At a minimum, this includes the analysis of QC samples including a method blank and a laboratory control sample (LCS) in each analytical batch, the addition of surrogates to each field sample and QC sample, and routine analyses of matrix spike and matrix spike duplicate aliquots.

8.4.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, the laboratories should use a matrix spike and matrix spike duplicate pair.

8.4.2 In-house method performance criteria should be developed using the guidance found in Sec. 8.0 of Method 8000 for procedures for evaluating method performance.

8.4.3 A laboratory control sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicates a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

8.4.4 Include a calibration standard after each group of 20 samples in the analysis sequence as a calibration check (it is recommended that a calibration standard be included after every 10 samples to minimize the number of repeat injections). Thus, injections of method blank extracts, matrix spike samples, and other non-standards are counted in the total. Solvent blanks, injected as a check on cross-contamination, need not be counted in the total. The response factors for the calibration should be within ±15% of the initial calibration. When this calibration verification standard falls out of this acceptance window, the laboratory should stop analyses and take corrective action.

9.0 METHOD PERFORMANCE

9.1 Table 1 lists example method detection limits (MDLs) for 10 target compounds in water and soil, developed using the optional sample extraction and cleanup procedures described in Sec. 7. Seven or ten replicate spiked samples were analyzed, as indicated in the table. The data are taken from References 1 and 6. The MDL is defined in Chapter One. **The MDLs listed in Table 1 were obtained using spiked organic-free reagent water and spiked clean soil and are provided for illustrative purposes only.** Each laboratory should develop its own matrix-specific MDLs, if necessary, using the guidance found in Chapter One.

9.2 Tables 2, 3, and 4 list the single-operator average recoveries and standard deviations for organic-free reagent water, wastewater, and soil prepared using the optional sample extraction and cleanup procedures described in Sec. 7. Ten replicate samples were analyzed for each matrix type. The data are taken from References 1 and 6.

9.3 Tables 5 and 6 provide single-operator accuracy (as recovery) and precision (as RSD) data for 14 carbamates spiked into bulk quantities of a POTW effluent and a soil. The spiking levels were based on the Universal Treatment Standard (UTS) values for wastewater and non-wastewater. The spiking levels were approximately 80% of the UTS level for the matrix, rounded to two significant figures. Four replicate samples of each matrix were extracted and analyzed. The wastewater samples were prepared by extracting a 1-L sample using continuous liquid-liquid extraction (Method 3520). The soil samples were prepared by extracting a 30-g sample using Soxhlet extraction (Method 3540). Four of the compounds eluted together in a single peak (bendiocarb, thiodicarb, carbofuran, and propoxur) and one pair of compounds eluted together in another peak (oxamyl and formentanate hydrochloride). The data are summarized in detail in Reference 7.

10.0 REFERENCES

1. California Department of Health Services, Hazardous Materials Laboratory, "*N*-Methylcarbamates by HPLC", Revision No. 1.0, September 14, 1989.

2. Krause, R.T. Journal of Chromatographic Science, 1978, vol. 16, pg 281.

3. Klotter, Kevin, and Robert Cunico, "HPLC Post Column Detection of Carbamate Pesticides", Varian Instrument Group, Walnut Creek, CA 94598.

4. USEPA, "Method 531. Measurement of *N*-Methylcarbomyloximes and *N*-Methylcarbamates in Drinking Water by Direct Aqueous Injection HPLC with Post Column Derivatization", EPA 600/4-85-054, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

5. USEPA, "Method 632. The Determination of Carbamate and Urea Pesticides in Industrial and Municipal Wastewater", EPA 600/4-21-014, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

6. Okamoto, H.S., D. Wijekoon, C. Esperanza, J. Cheng, S. Park, J. Garcha, S. Gill, K. Perera "Analysis for *N*-Methylcarbamate Pesticides by HPLC in Environmental Samples", Proceedings of the Fifth Annual USEPA Symposium on Waste Testing and Quality Assurance, July 24-28, 1989, Vol. II, 57-71.

7. “Carbamates Method Evaluation Report,” report for EPA Contract 68-W6-0068, Science Applications International Corporation, Dunn Loring, VA 22027, August 25, 1998.

TABLE 1

EXAMPLE ELUTION ORDER, RETENTION TIMES AND METHOD DETECTION LIMITS

		Method Detection Limits[a]	
Compound	Retention Time (min)	Reagent Water (µg/L)	Soil (µg/kg)
Aldicarb sulfone	9.59	1.9[b]	44[b]
Methomyl	9.59	1.7	12
3-Hydroxycarbofuran	12.70	2.6	10[b]
Dioxacarb	13.50	2.2	>50[b]
Aldicarb	16.05	9.4[b]	12[b]
Propoxur	18.06	2.4	17
Carbofuran	18.28	2.0	22
Carbaryl	19.13	1.7	31
α-Naphthol[c]	20.30	-	-
Methiocarb	22.56	3.1	32
Promecarb	23.02	2.5	17

See Sec. 7.6 for chromatographic conditions. Retention times are provided for illustrative purposes only. Each laboratory must determine retention times and retention time widows for their specific application of the method.

[a] MDLs for organic-free reagent water and soil were determined by analyzing 10 low concentration spiked replicates for each matrix type (except where noted). See References 1 and 6 for more details. These MDLs were obtained using spiked organic-free reagent water and clean soil, using the optional sample extraction and cleanup procedures described in Sec. 7, and are provided for illustrative purposes only.

[b] MDL determined by analyzing 7 spiked replicates.

[c] Breakdown product of Carbaryl.

TABLE 2

SINGLE-OPERATOR RECOVERY AND PRECISION DATA FOR SPIKED REAGENT WATER

Compound	Concentration (µg/L)	% Recovery	% RSD
Aldicarb sulfone	225	75	3.2
Methomyl	244	81	3.4
3-Hydroxycarbofuran	210	70	3.7
Dioxacarb	241	80	3.5
Aldicarb	224	75	6.0
Propoxur	232	77	4.6
Carbofuran	239	80	3.9
Carbaryl	242	81	3.5
Methiocarb	231	77	3.5
Promecarb	227	76	4.1

Spiking concentration = 300 µg/L of each compound

n = 10

The data are taken from References 1 and 6.

TABLE 3

SINGLE-OPERATOR RECOVERY AND PRECISION DATA FOR SPIKED WASTEWATER

Compound	Concentration (µg/L)	% Recovery	% RSD
Aldicarb sulfone	235	78	7.5
Methomyl	247	82	12.1
3-Hydroxycarbofuran	251	84	10.1
Dioxacarb	NR	-	-
Aldicarb	258	86	6.4
Propoxur	263	88	6.5
Carbofuran	262	87	6.0
Carbaryl	262	87	6.6
Methiocarb	254	85	7.8
Promecarb	263	88	5.7

Spiking concentration = 300 µg/L of each compound

n = 10

NR = No recovery

The data are taken from References 1 and 6.

TABLE 4

SINGLE-OPERATOR RECOVERY AND PRECISION DATA FOR SPIKED SOIL

Compound	Concentration (mg/kg)	% Recovery	% RSD
Aldicarb sulfone	1.57	79	4.4
Methomyl	1.48	74	5.8
3-Hydroxycarbofuran	1.60	80	4.4
Dioxacarb	1.51	76	4.8
Aldicarb	1.29	65	11.0
Propoxur	1.33	67	9.5
Carbofuran	1.46	73	6.3
Carbaryl	1.53	77	4.9
Methiocarb	1.45	73	4.9
Promecarb	1.29	65	9.6

Spiking concentration = 2.00 mg/kg of each compound

n = 10

The data are taken from References 1 and 6.

TABLE 5

SINGLE-LABORATORY RECOVERY AND PRECISION DATA FOR ANALYSIS OF CARBAMATES EXTRACTED FROM WASTEWATER BY CONTINUOUS LIQUID-LIQUID EXTRACTION (METHOD 3520)

Compound	Spiking Level (µg/L)	Mean % Recovery	% RSD
Aldicarb sulfone	45	42	6.3
Bendiocarb	45	88*	8.6
Carbaryl	5	84	7.2
Carbofuran	5	88*	8.6
m-Cumenyl-methylcarbamate	45	79	7.8
Formetanate hydrochloride	45	43**	6.7
Methiocarb	45	83	9
Methomyl	22	66	3.2
Metolcarb	45	86	6.4
Mexacarbate	45	70	13.3
Oxamyl	45	43**	6.7
Promecarb	45	82	9
Propoxur	45	88*	8.6
Thiodicarb	15	88*	8.6

The asterisks indicate compounds that coelute on the HPLC column. Those marked with one asterisk (*) represent one group of compounds that elute together, those with two asterisks (**) are a second group. The reported recoveries and RSD values are based on the total concentration of all of the compounds that coeluted.

Mean recoveries and RSDs are calculated from the extraction and analysis of four replicate samples. 1-L samples were extracted.

The data are taken from Reference 7.

TABLE 6

SINGLE-LABORATORY RECOVERY AND PRECISION DATA FOR ANALYSIS OF CARBAMATES EXTRACTED FROM SOIL BY SOXHLET EXTRACTION (METHOD 3540)

Compound	Spiking Level (µg/kg)	% Mean Recovery	% RSD
Aldicarb sulfone	2200	78	5.6
Bendiocarb	1100	83*	5.8
Carbaryl	110	107	5.1
Carbofuran	110	83*	5.8
m-Cumenyl-methylcarbamate	1100	77	4.9
Formetanate hydrochloride	1100	73**	6.2
Methiocarb	1100	80	6.3
Metolcarb	1100	84	6.8
Methomyl	110	0	0
Mexacarbate	1100	71	14.8
Oxamyl	2200	73**	6.2
Promecarb	1100	82	5.6
Propoxur	1100	83*	5.8
Thiodicarb	1100	83*	5.8

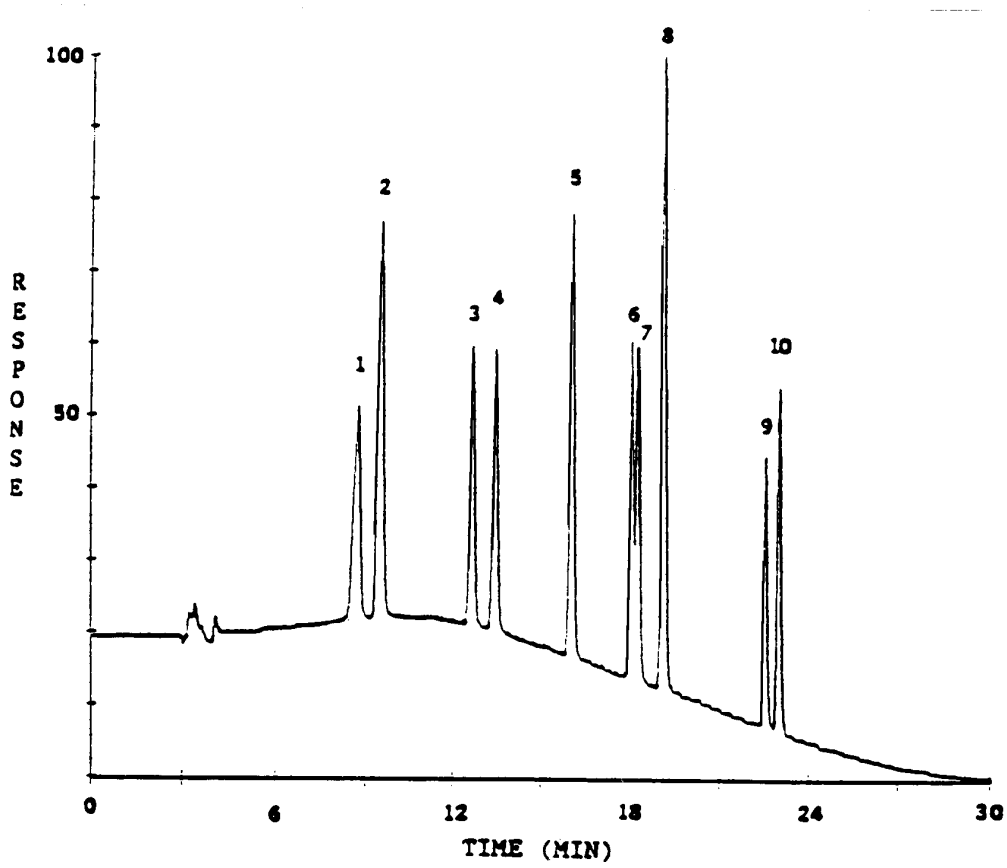
The asterisks indicate compounds that coelute on the HPLC column. Those marked with one asterisk (*) represent one group of compounds that elute together, those with two asterisks (**) are a second group. The reported recoveries and RSD values are based on the total concentration of all of the compounds that coeluted.

Mean recoveries and RSDs are calculated from the extraction and analysis of four replicate samples. 30-g samples were extracted.

The data are taken from Reference 7.

FIGURE 1

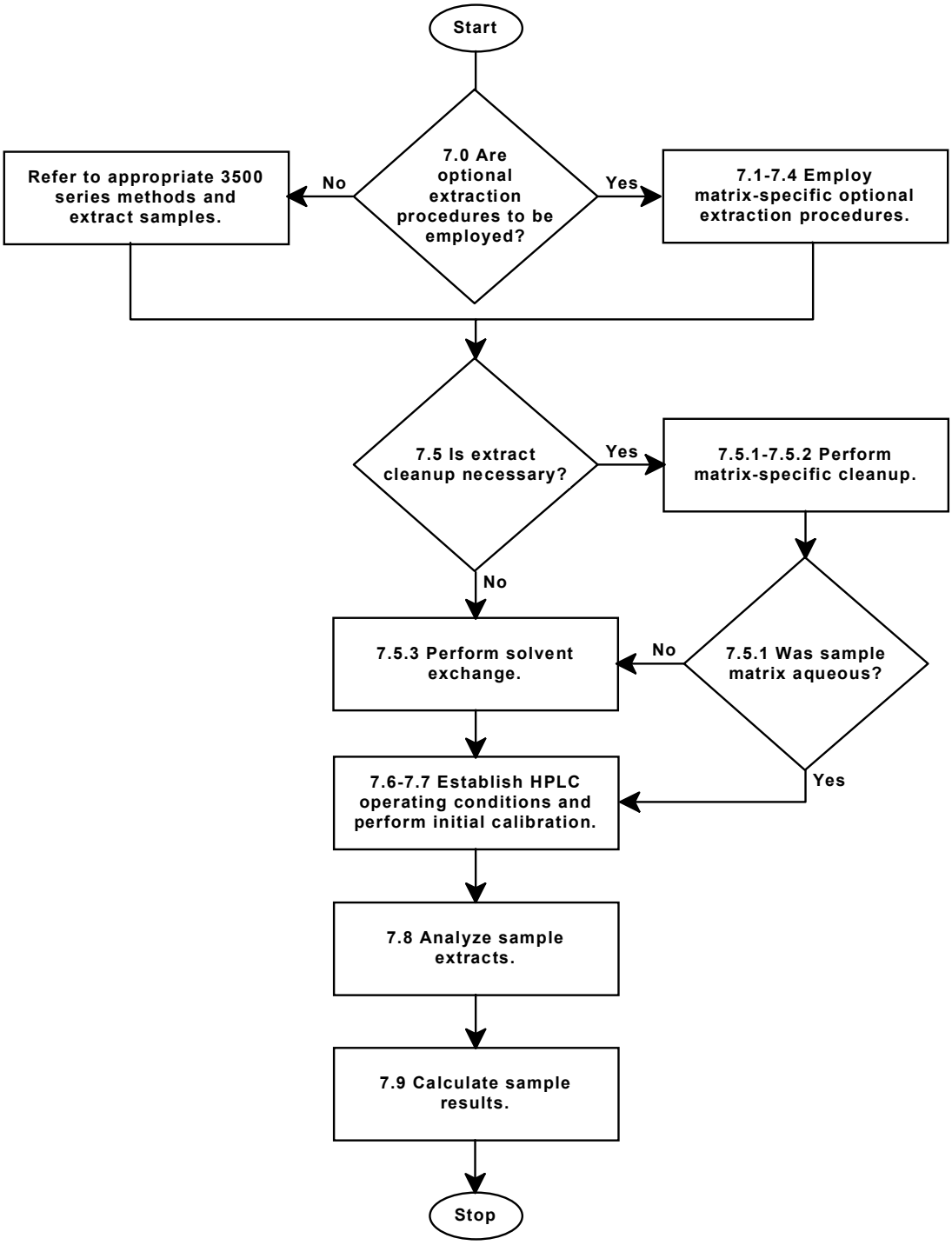
EXAMPLE SEPARATION OF 10 OF THE TARGET COMPOUNDS



1.00 mg/mL each of:

1. Aldicarb sulfone
2. Methomyl
3. 3-Hydroxycarbofuran
4. Dioxacarb
5. Aldicarb
6. Propoxur
7. Carbofuran
8. Carbaryl
9. Methiocarb
10. Promecarb

This chromatogram was produced using the instrument operating conditions described in Sec. 7 and is for illustrative purposes only.


Start
7.0 Are optional extraction procedures to be employed?
No
Refer to appropriate 3500 series methods and extract samples.
Yes
7.1-7.4 Employ matrix-specific optional extraction procedures.
7.5 Is extract cleanup necessary?
Yes
7.5.1-7.5.2 Perform matrix-specific cleanup.
No
7.5.1 Was sample matrix aqueous?
No
7.5.3 Perform solvent exchange.
Yes
7.6-7.7 Establish HPLC operating conditions and perform initial calibration.
7.8 Analyze sample extracts.
7.9 Calculate sample results.
Stop